

## Fate of ivermectin residues in ewes' milk and derived products

Vesna Cerkvenik<sup>1\*</sup>, Bogdan Perko<sup>2</sup>, Irena Rogelj<sup>2</sup>, Darinka Z Doganoc<sup>1</sup>, Valentin Skubic<sup>3</sup>, Wim MJ Beek<sup>4</sup> and Henk J Keukens<sup>5</sup>

<sup>1</sup> Institute of Food Hygiene and Bromatology, Veterinary Faculty of the University of Ljubljana, Gerbičeva 60, SI – 1000 Ljubljana, Slovenia

<sup>2</sup> Institute of Dairying, Zootechnical Department, Biotechnical Faculty of the University of Ljubljana, Groblje 3, SI – 1230 Domžale, Slovenia

<sup>3</sup> Institute of Physiology, Pharmacology and Toxicology, Veterinary Faculty of the University of Ljubljana, Gerbičeva 60, SI – 1000 Ljubljana, Slovenia

<sup>4</sup> RIKILT, State Institute for Quality Control of Agricultural Products, Bornsesteeg 45, NL – 6700 AE Wageningen, Netherlands

<sup>5</sup> Laboratory of the National Meat and Inspection Service, P.O. Box 144, NL – 6700 AC Wageningen, Netherlands

Received 1 March 2002 and accepted for publication 30 April 2003

The fate of ivermectin (IVM) residues was studied throughout the processing of daily bulk milk from 30 ewes (taken up to 33 d following subcutaneous administration of 0.2 mg IVM/kg b.w.) in the following milk products: yoghurt made from raw and pasteurized milk; cheese after pressing; 30- and 60-day ripened cheese; and whey, secondary whey and whey proteins obtained after cheese-making (albumin cheese). The concentration of the H<sub>2</sub>B<sub>1a</sub> component of IVM was analysed in these dairy products using an HPLC method with fluorescence detection. The mean recovery of the method was, depending on the matrix, between 87 and 100%. Limits of detection in the order of only 0.1 µg H<sub>2</sub>B<sub>1a</sub>/kg of product were achieved. Maximum concentrations of IVM were detected mostly at 2 d after drug administration to the ewes. The highest concentration of IVM was found on day 2 in 60-day ripened cheese (96 µg H<sub>2</sub>B<sub>1a</sub>/kg cheese). Secondary whey was the matrix with the lowest concentration of IVM (<0.6 µg H<sub>2</sub>B<sub>1a</sub>/kg). Residue levels fell below the limits of detection between day 5 (for secondary whey) and day 25 (for all cheese samples). In the matrices investigated, linear correlations between daily concentrations of IVM, milk fat and solid content were evident. During yoghurt production, fermentation and thermal stability of IVM was observed. During cheese production, approximately 35% of the IVM, present in the raw (bulk) milk samples, was lost. From the results it was concluded that the processing of ewes' milk did not eliminate the drug residues under investigation. The consequences of IVM in the human diet were discussed. Milk from treated animals should be excluded from production of fat products like cheese for longer after treatment with IVM than for lower fat products.

**Keywords:** Ivermectin, ewes, residues, milk, products.

Parasitic diseases are among the most severe problems in veterinary medicine. They cause severe economic losses due to damage to the health of infected animals. For this reason intensive breeding cannot avoid contemporary antiparasitic programmes. In recent years the development of antiparasitic drugs has been rapid, reflected by an increase in the market of 70% in EU countries in the 1989–1995 period (FEDESA, 1997). Avermectins and milbemycins represent the greatest progress in antiparasitic therapy since 1981, when ivermectin (IVM), as the first, was introduced

into veterinary medicine. These substances have shown unique activity against a broad spectrum of both endo- and ectoparasites in extremely low doses (Benz et al. 1989; Courtney & Roberson, 1995). They are very soluble in body fats (Lo et al. 1985) and these characteristics are the reason for their prolonged efficacy. On the other hand, the long retention of these drug residues in food-producing animals is problematic due to their long excretion time from the organism and the consequent contamination of food, including milk.

This paper deals with residues of IVM, the leading veterinary drug in sales worldwide (Miller, 1993). IVM consists of 2 closely related components – homologues

\* For correspondence; e-mail: Vesna.Cerkvenik@vf.uni-lj.si

containing not less than 80% of 22,23-dihydroavermectin B<sub>1a</sub> (H<sub>2</sub>B<sub>1a</sub>) and not more than 20% of 22,23-dihydroavermectin B<sub>1b</sub> (H<sub>2</sub>B<sub>1b</sub>) (The Merck Index, 1989). In terms of concentration and excretion time the major component is the marker substance for IVM in foodstuffs of animal origin.

Various injectable formulations of IVM are also registered in Slovenia for the treatment and prophylaxis of sheep, excluding the lactation period, when the use of IVM is prohibited. Treatment with IVM is banned at least 21–33 d before lambing. But for some, even accidental reasons (Davis, 1991), IVM use with milking animals cannot always be avoided. Instructions for its use clearly state that the withdrawal time for milk has not been determined. During our previous experiment with sheep treated subcutaneously with IVM, a long excretion time (a mean of 23 d) of IVM into the milk was recorded (Cerkvenik et al. 2002). This fact is important from both health and economic aspects.

The majority of ewes' milk in Slovenia is not consumed as drinking milk but is processed into milk products, mostly cheese. The main purpose of conducting the experiments described was to examine the distribution and stability of IVM residues in ewes' milk submitted for processing. The thermisation of ewes' milk during pasteurization (74 °C, 40 s), high pasteurization (80 °C, 1 min) and boiling (100 °C, 10 s) did not influence IVM residue concentrations (Cerkvenik et al. 2001). Because of the sensitivity of IVM to acidic conditions (Downing, 1989), it was possible that the substance could be partly or completely degraded during lactic acid fermentation.

To our knowledge there is only one analytical method in the literature regarding the quantification of IVM residues in cheese i.e. in Mozzarella cheese from buffalo milk (Esposito et al. 2001), but no analytical approach has been published to quantify IVM residues in other milk products such as yoghurt, whey, albumin and hard-ripened cheese. Our second purpose was thus to introduce and validate analytical procedures that would enable us to answer the main question about the fate of IVM residues in ewes' milk products. Finally, the residue aspects of IVM regarding safety or hazard of milk products for the consumer were discussed.

This work is part of a complete study of IVM stability in different ewes' milk products (Cerkvenik, 2000).

## Materials and Methods

### Milk samples

Thirty ewes of milking crossbreed in the highest lactation period (just after weaning), kept indoors, were used in the experiment. They were given Ivomec® (MSD AGVET) subcutaneously in a single dose of 0.2 mg IVM/kg b.w. The bulk milk obtained from an evening and morning milking of all animals was used for processing. The first milk sample was taken before IVM administration. After IVM

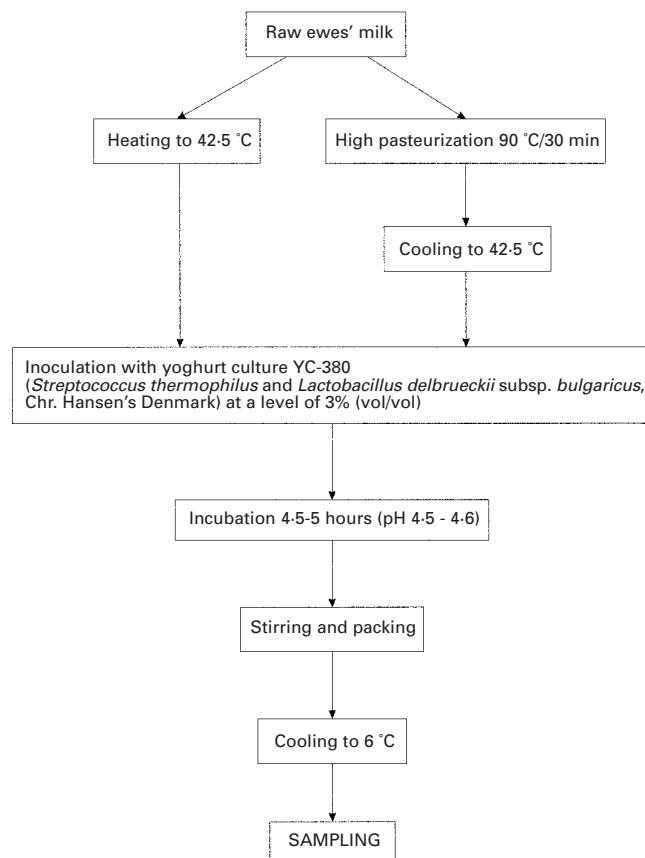


Fig. 1. Protocol for the manufacture of yoghurt.

administration, milk was collected on days 1, 2, 3, 4, 5, 7, 9, 12, 15, 17, 21, 25, 29, and 33. In total, 15 daily bulk milk samples were processed during the experiment. An aliquot of each sample was always taken before milk processing and was kept frozen at –20 °C until the analysis.

### Milk products samples

From daily bulk milk samples collected during the experiment, the following products were made: yoghurt from raw milk, yoghurt from pasteurized milk, semi-hard cheese and albumin cheese. Technological protocols and sampling are presented in Figs 1 and 2.

In total, 114 samples were taken and analysed. Samples were stored at –20 °C before analysis.

### Determination of IVM

Pure standard in glycerol was obtained as a gift from the RIKILT institute in Wageningen (The Netherlands). Standard contained 1390 mg H<sub>2</sub>B<sub>1a</sub>/100 g and 110 mg H<sub>2</sub>B<sub>1b</sub>/100 g. Standardized solutions were prepared in acetonitrile.

Reagents were obtained from Merck (Darmstadt, Germany) and were of p.a. purity, but for preparation of the mobile phase, LiChrosolv solvents were used. Extraction

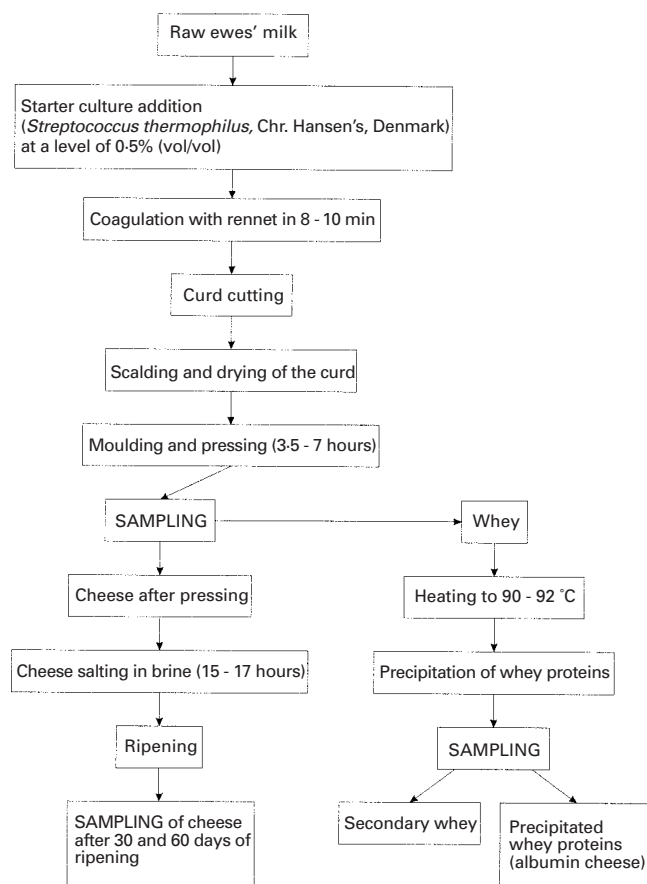


Fig. 2. Protocol for the manufacture and sampling of cheese.

columns Bakerbond with  $C_8$  sorbent (500 mg, 6 ml) were purchased from J. T. Baker, Phillipsburg, NJ, USA (Cat. No. 7087-06).

The concentration of the  $H_2B_{1a}$  component in ewes' milk and milk products was determined using a combined HPLC method with fluorescence detection (De Montigny et al. 1990; Nordlander & Johnsson, 1990; Beek, 1992). Samples (5 g) were extracted with 20 ml acetonitrile. Manual shaking for 5 min, ultrasound for 15 min (ultrasonification bath Iskra: UZ 4P and Sonis 4, Šentjernej, Slovenia) and manual shaking again for 5 min were used for extraction of IVM from milk, yoghurt, whey and albumin cheese. A sample blender (Iskra Prins, Ljubljana, Slovenia) was used 3 min for extraction from cheese after pressing, and also from ripened cheese. After centrifugation at  $3290 \times g$ , for 10 min (centrifuge Heraeus: Minifuge T, Osterode, Germany), 50  $\mu$ l triethylamine was added to 15 ml supernatant. The mixture was diluted with water to 50 ml and cleaned-up using a solid phase extraction on  $C_8$  cartridges. After applying the extract, the columns were washed with 5 ml of a mix of acetonitrile, water and triethylamine (50:50:0.1, v/v/v). Eluate (5 ml, in acetonitrile) was concentrated at 50 °C under a nitrogen stream (evaporator Organomation: N-evap No 111, Berlin, MA, USA and Liebsch: 2366, Bielefeld, Germany) and dry

extracts were derivatized at room temperature with 100  $\mu$ l *N*-methylimidazole solution in acetonitrile (1:1, v/v) and 150  $\mu$ l trifluoroacetic anhydride solution in acetonitrile (1:2, v/v). After 30 s acetonitrile was added to the formed conjugated fluorescent derivative to obtain a final volume of 1 ml.

Fifty microlitres of the diluted fluorescent derivative was injected into the liquid chromatograph and the results were evaluated according to the external standard method. HPLC system consisted of a Perkin Elmer LC-10 pump module (Norwalk, Connecticut, USA), Rheodyne 7125 injector with a 50  $\mu$ l loop (Cotati, CA, USA), a Chrompack M 57020-88-2 thermostat for precolumn and analytical column (Middelburg, The Netherlands), a Shimadzu RF-535 fluorescence detector (Kyoto, Japan), a Hewlett Packard 35900 A/D converter (USA) and a Hewlett Packard HP 3365 ChemStation integration software (USA). The chromatographic process was performed at 27 °C on Supelco's Supelcosil LC-8-DB  $150 \times 4.6$  mm (5  $\mu$ m) reversed deactivated analytical column with a 2 cm pre-column filled with the same stationary phase. The mobile phase was a mix of acetonitrile:methanol:water (475:475:50, v/v/v) and was pumped at a flow rate of 1.1 ml/min. Excitation and emission wavelengths were 364 and 470 nm respectively.

#### Validation of the analytical method of IVM determination

Extensive validation of the method was performed for each matrix with the use of spiked blank materials in concentration regions similar to the samples taken. Selectivity was evaluated by comparing the chromatograms of the blank samples with spiked samples. Linearity was expressed by correlation between chromatographic peak areas and concentrations for standards at a concentration range between 600 and 0.75 ng  $H_2B_{1a}$ /ml. Measurements were performed on three separate days to evaluate recovery, with three fortification levels in five replicates on each day. The repeatability of the method was evaluated in parallel with recovery tests and was expressed using a recovery coefficient of variation. The limit of detection of standards and matrices was determined according to signal to noise ratio of a value of 3. Limit of quantification (LOQ) was formulated to be the lowest analyte content for which the method has been validated with specified degrees of accuracy and repeatability. Stability was evaluated for the solid phase extraction eluates in acetonitrile during storage at 4 °C, as well as for the derivatized standards and samples.

#### Determination of fat and solid contents

Fat and solid contents were determined using the Gerber acidobuthyrometric method and drying to constant mass respectively (Roeder, 1954). Whey samples were an exception, in which solid content was analysed by refractometry (Bradley et al. 1992).

### Stability of IVM during milk processing

Stability of IVM during milk processing (Figs 1 and 2) was evaluated for the first 12 d after drug administration to the ewes as described above.

Influence of lactic acid fermentation (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) was studied by comparing daily IVM concentrations in yoghurt made from raw milk and raw – unpasteurized (bulk) milk. Influence of thermal treatment (heating at 90 °C for 30 min) was studied by comparing daily IVM concentrations in yoghurt made from pasteurized milk and raw (bulk) milk. Influence of cheese-making processes (including fermentation with *Strep. thermophilus*, heating, renneting, salting and ripening) was studied by comparing the IVM content (concentration multiplied by mass) in the cheese products (cheese, whey) and raw (bulk) milk dedicated for the production of cheese products.

## Results and Discussion

### Validation of the analytical method

For the determination of IVM residues in milk products, a previously used analytical method for milk (Cerkvenik et al. 2001) was successfully adapted, with some modifications, during our experiment. To estimate the content of IVM in the samples, its main component ( $H_2B_{1a}$ ) was quantified, although the analytical method can also detect the minor component ( $H_2B_{1b}$ ). After use of a sample blender, 14% higher concentrations of IVM were detected in cheese after pressing in comparison with ultrasound extraction.

The validation parameters achieved proved the suitability of the analytical method for use in the planned experiment. The method used was selective. No interferences of endogenous substances or impurities resulting from the analytical procedure were present at the retention time of the  $H_2B_{1a}$ . A clear background on the wider area around the  $H_2B_{1a}$  retention time was also obtained, with the exception of ripened cheese, where interferences increased together with the ripening time. For this reason, the intervals between injections were longer for ripened cheese compared with other matrices. The mean correlation coefficient for standards at a concentration range between 600 and 0.75 ng  $H_2B_{1a}$ /ml was 0.9999. The mean recovery obtained was, depending on the matrix, between 86.9% for cheese after pressing and 100.3% for cheese ripened for 60 d (Table 1). Coefficient of the recovery (=repeatability) was below 10% (Table 1). The limit of detection was 0.3 ng  $H_2B_{1a}$ /ml (15 pg/50 µl injection loop) for standards, while in matrices it differed according to fat and solid content (0.08 µg  $H_2B_{1a}$ /kg cheese, 0.1 µg  $H_2B_{1a}$ /kg yoghurt or whey). Limits of quantification were approximately one order of magnitude higher than detection limits and were for cheese 0.15 µg  $H_2B_{1a}$ /kg (mean recoveries at this concentration level were between 94.6

**Table 1.** Recovery tests for ivermectin (IVM) determination in ewes' milk products. Blank samples were spiked with three concentrations of  $H_2B_{1a}$  on three consecutive days (daily each concentration in five parallels)

Values for each concentration are means of  $n=15$

Matrix	Spiked (µg/kg)	Recovery (%) Mean (CV)
Yoghurt made from raw milk	2	103.42 (9.5)
	10	97.78 (2.2)
	20	97.44 (3.0)
	Overall	99.55 (6.1)
Yoghurt made from pasteurized milk	2	97.31 (9.1)
	10	98.35 (1.9)
	20	99.10 (3.2)
	Overall	98.25 (5.6)
Cheese after pressing	5	83.81 (5.1)
	25	88.42 (3.8)
	50	88.41 (2.6)
	Overall	86.88 (3.9)
Cheese ripened 30 days	5	95.34 (2.9)
	25	97.15 (1.9)
	50	95.43 (2.3)
	Overall	95.97 (2.4)
Cheese ripened 60 days	5	98.22 (3.7)
	25	99.37 (2.7)
	50	103.35 (2.3)
	Overall	100.31 (3.0)
Whey	0.5	96.76 (5.1)
	1.25	97.77 (9.0)
	2.5	97.76 (8.8)
	Overall	97.43 (7.9)
Secondary whey	0.25	96.88 (5.7)
	0.5	101.74 (3.5)
	1	100.85 (3.6)
	Overall	99.89 (4.3)
Albumin cheese	2	100.09 (2.9)
	10	95.58 (3.0)
	20	90.69 (5.2)
	Overall	95.45 (3.8)

and 104.0% and CVs of recovery were between 1.9 and 5.2%) and for yoghurt and whey 0.2 µg  $H_2B_{1a}$ /kg (mean recoveries at this concentration level were between 93.7 and 108.5% and CVs of recovery were between 3.5 and 12.1%). The validation results for ewes' milk have already been presented (Cerkvenik et al. 2001). Solid phase extraction eluates in acetonitrile were completely stable during three-day storage at 4 °C, while the high stability of the derivatized standards and samples (kept in darkness) was limited to 1.5–2.5 h respectively due to hydrolysis of the fluorescent IVM derivative.

### IVM residues and their stability during milk processing

Due to its lipophilicity IVM was substantially excreted into milk of subcutaneously treated ewes and residence time of the found residues was long. Time profile of IVM concentrations in the raw (bulk) milk samples of a group of

**Table 2.** Ivermectin (IVM) concentrations ( $\mu\text{g H}_2\text{B}_{1a}/\text{kg}$ ) in daily bulk milk samples of 30 ewes and milk products 0–33 d after s.c. administration of 0.2 mg IVM/kg b.w.

Values are means of $n=2$									
Day	Bulk milk	Yoghurt		Cheese			Whey		Albumin cheese
		from raw milk	from pasteurized milk	after pressing	ripened		secondary		
					30 d	60 d			
0	<0.15	—	<0.1	<0.08	<0.08	<0.08	—	—	<0.08
1	21.91	—	22.13	58.86	67.62	82.46	2.87	0.56	46.79
2	24.26	22.07	22.82	68.90	75.06	96.11	2.51	0.29	30.52
3	18.40	15.34	18.12	42.75	67.55	71.27	1.87	0.24	25.59
4	13.39	12.29	14.17	35.54	50.13	56.46	1.59	—	19.14
5	10.50	9.01	10.21	26.18	38.63	42.08	1.27	<0.1	16.29
7	4.75	5.23	5.25	12.55	24.35	22.99	0.62	<0.1	6.49
9	3.16	3.18	3.12	8.79	14.75	14.32	<0.1	<0.1	4.64
12	1.22	1.20	1.19	2.61	5.18	5.78	0.16 <sup>a</sup>	<0.1	1.91
15	0.59	0.53	0.56	1.63	2.48	2.52	<0.1	<0.1	1.10
17	0.33	0.28	0.30	0.72	1.14	1.41	<0.1	<0.1	0.56
21	<0.15	<0.1	<0.1	0.22	0.38	0.44	<0.1	<0.1	0.14 <sup>a</sup>
25	<0.15	<0.1	<0.1	<0.08	<0.08	<0.08	<0.1	<0.1	<0.08
29	<0.15	—	<0.1	<0.08	<0.08	<0.08	<0.1	<0.1	<0.08
33	<0.15	<0.1	<0.1	<0.08	<0.08	<0.08	<0.1	<0.1	<0.08

<sup>a</sup> between limit of detection and limit of quantification (semi-quantitative estimate)

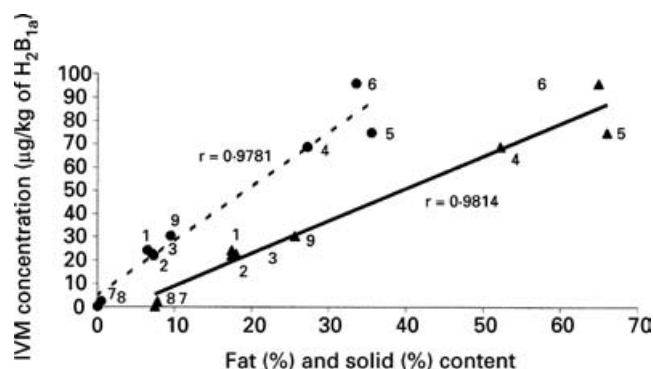
&lt; below limit of detection

— milk product was not produced

30 ewes, given a single dose of 0.2 mg IVM/kg b.w. is presented in Table 2 and it can be seen that it essentially determined the subsequent levels in the derived milk products. The maximum concentration of 24.3  $\mu\text{g H}_2\text{B}_{1a}/\text{kg}$  in raw (bulk) milk appeared on the second day after IVM administration into the ewes and was a little above a maximum mean daily value for a group of six ewes in the experiment described by Cerkvenik et al. (2002). IVM concentrations in the raw (bulk) milk fell below the limit of detection of the analytical method after 21 d, which was also in accordance with our previous investigations (Cerkvenik et al. 2002) and gives an indication of the longevity of IVM in the system after the drug administration.

The fate of IVM residues during ewes' milk processing was studied, including lactic acid fermentation, heating, renneting, salting and ripening (for cheese). Milk products were chosen according to the production in praxis, both industrial and domestic. Because yoghurt from raw (unpasteurized) milk is still common among small private producers, yoghurt made from both raw and pasteurized milk was included in the study.

IVM residue concentrations in the derived milk products during the experiment are also shown in Table 2. Concentration-time profiles for yoghurt made from raw and pasteurized milk were similar to the concentration-time profile for the raw (bulk) milk. Concentration-time curves for the cheese products differed significantly, mostly according to the fat and solid content in these matrices. A

**Fig. 3.** Ivermectin (IVM) residues in daily bulk milk samples and milk products on the second day of the experiment (after s.c. administration of 0.2 mg IVM/kg b.w. to 30 ewes) in relation to milk fat (---) and solid content (—) in these matrices.

Marks:

1 – bulk milk; 2 – yoghurt made from raw milk; 3 – yoghurt made from pasteurized milk; 4 – cheese after pressing; 5 – 30-day ripened cheese; 6 – 60-day ripened cheese; 7 – whey; 8 – secondary whey; 9 – albumin cheese.

linear correlation between daily IVM concentrations in the matrices investigated and milk fat and solid content in these matrices was evident (Fig. 3), and can be explained due to the lipophilicity of the drug (Lo et al. 1985).

IVM concentration ratios in milk products remained mostly the same at day 2, 5 and 15 after the IVM administration. The highest IVM residue content was found in

**Table 3.** Ivermectin (IVM) stability (%) during ewes' milk processing – influence of lactic acid fermentation (yoghurt made from raw milk), thermal treatment (yoghurt made from pasteurized milk) and cheese-making processes. Calculations were performed for the first 12 d after s.c. administration of 0.2 mg IVM/kg b.w. to 30 ewes; daily bulk milk samples were processed

Day	Yoghurt made from		Cheese-making processes					Cheese products: cheese+whey
	Raw milk	Pasteurized milk	Bulk milk†	Cheese (all sorts together)		Whey		IVM stability
				IVM content		IVM content		
	%‡	%§	µg H <sub>2</sub> B <sub>1a</sub>	µg H <sub>2</sub> B <sub>1a</sub>	%¶	µg H <sub>2</sub> B <sub>1a</sub>	%††	%¶ + %††
1	—	101·0	431·6	177·7	41·2	45·9	10·6	51·8
2	91·0	94·1	477·9	250·7	52·5	40·2	8·4	60·9
3	83·4	98·5	323·8	172·4	53·2	26·2	8·1	61·3
4	91·8	105·8	207·5	128·3	61·8	19·1	9·2	71·0
5	85·8	97·2	174·3	101·6	58·3	16·5	9·5	67·8
7	110·1	110·5	93·6	60·8	65·0	9·9	10·6	75·6
9	100·6	98·7	52·5	36·0	68·6	1·7	3·2	71·8
12	98·4	97·5	21·5	11·5	53·5	2·2	10·2	63·7
Mean	94·4	100·4			56·8		8·7	65·5

— milk product was not produced

† milk dedicated for cheese production

‡ daily IVM concentration ratio yoghurt from raw milk/bulk milk × 100 (%)

§ daily IVM concentration ratio yoghurt from pasteurized milk/bulk milk × 100 (%)

¶ daily IVM content (= concentration × mass) ratio of cheese/bulk milk dedicated for cheese production × 100 (%)

†† daily IVM content (= concentration × mass) ratio of whey/bulk milk dedicated for cheese production × 100 (%)

60-day ripened cheese (96.1 µg H<sub>2</sub>B<sub>1a</sub>/kg) and the lowest in secondary whey, where concentrations were below 0.6 µg H<sub>2</sub>B<sub>1a</sub>/kg for the entire time of experiment. Following the raw (bulk) milk concentration levels, in all matrices investigated, maximum IVM concentrations were found on the second day, with the exception of whey proteins (albumin cheese). In our opinion the sharp decrease of fat content from 14 to 10% from the first to the second day can be the reason that, in this matrix, maximum IVM concentrations appeared on the first day of the experiment. IVM residue content fell below the detection limit of the analytical method from day 5 after drug administration to the ewes for secondary whey, and day 25 for cheese samples, which reflected the excretion time of the drug in the raw (bulk) milk.

Residues of IVM were stable during lactic acid fermentation and thermal treatment (Table 3). After the fermentation of milk with *Strep. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*, the pH reached the value around 4–4.6. The main factor which lowered the pH value was lactic acid which is a weak acid and as such unable to cause the hydrolysis of IVM. It is well known that strong acids such as sulphuric acid, by giving much lower pH value, cause the acid hydrolysis of IVM by separation of one or two sugar units, which leads to transformation and (partly) deactivation of the drug to monosaccharide or aglycone (Downing, 1989; FSIS, 1991). IVM was also sustainable against high pasteurization in the production of yoghurt, too. High thermal stability of IVM residues was also in agreement with our previous findings (Cerkvenik

et al. 2001). About 35% of IVM residues were lost during cheese production (Table 3).

Till now the fate of veterinary drugs during milk processing has been studied only for cheese-making processes, with the production of yoghurt being excluded. IVM residues were studied only in Mozzarella cheese from buffalo milk (Esposito et al. 2001). After the same administration of IVM at the same milk collection time points Mozzarella cheese contained higher IVM concentrations than our cheese after pressing, which was a consequence of higher residue concentrations in the raw (bulk) buffalo milk than ewes' milk. The distribution of veterinary drug residues in milk products has been described also for albendazole (Longo et al. 1996; Fletouris et al. 1998) and ampicillin (Cinquina et al. 1999).

#### IVM residue legislation and health aspects

In Slovenia the residue legislation of veterinary medicinal products, including IVM is unified with European Union since 2000 (R. Slovenia, 2000). There are no Maximum Residue Levels (MRLs) for IVM in milk (bovine or ovine) in the EU regarding the Regulation (EEC) No 2377/90 (EEC, 1990). This means that IVM-based products may not be used in animals which are producing milk for human consumption and that residues in milk are not allowed (zero tolerance). However, in cases of emergency (other products not available, life-threatening situation for the animal, etc. – this might not always be the same in all EU Member States) it could mean that such a product can be

administered to milk-producing animals provided that the milk is kept out of the normal distribution channel for human consumption and the withdrawal period will have to be judged by the veterinarian.

The Acceptable Daily Intake (ADI) value for IVM residues is 60 µg per day for a standard 60 kg person, based on a No Observed Effect Level (NOEL) value in teratogenicity studies (WHO, 1993). This gives an indication for the 'seriousness' of residues. However, the ADI is set as a safe limit and any violation of that level is considered to be serious, independent from the type of effects studied/found in the toxicological studies. Based on very preliminary calculations and taking into account the MRLs set in the EU for ovine fat (20 µg/kg) and ovine liver (15 µg/kg) (EEC, 1993), it can be assumed that the ADI will be exceeded if levels of IVM in ovine milk will be in the magnitude of 30 µg/kg or higher and this must be considered to be unacceptable risk for consumer's health. Our results suggest that for this safety reason milk from treated animals should be excluded from production of fat products like cheese for longer after treatment with IVM than for lower fat products.

This study was supported by the Ministry of Science and Technology, and by the Ministry of Agriculture, Forestry and Food of the Republic of Slovenia. The authors also thank Mr K Zanuttini and his farm staff in Vremščica for maintaining the animals, MSc A Mijovič, DVM and S Penko, DVM for veterinary services, Mrs A Süssinger for analytical assistance and MSc Š Pintarič, DVM for graphic presentation. Dr JP Hoogland is sincerely thanked for the information about legislation and health aspects of IVM residues.

## References

- Beek WMJ 1992 [Determining ivermectin in muscle tissue, liver, kidney and milk by HPLC], pp. 6. Wageningen, The Netherlands: RIKILT – DLO
- Benz GW, Roncalli RA & Gross SJ 1989 Use of ivermectin in cattle, sheep, goats and swine. In *Ivermectin and Abamectin*, pp. 215–229 (Ed. WC Campbell). New York, USA: Springer-Verlag
- Bradley RL Jr, Arnold E Jr, Barbano DM, Semerad RG, Smith DE & Vines BK 1992 Chemical and physical methods. In *Standard Methods For The Examination Of Dairy Products*, 16th edn, pp. 493–494 (Ed. RT Marshall). Washington, DC: American Public Health Association
- Cerkvenik V 2000 [Residues of ivermectin in sheep plasma and milk, and the influence of thermal treatment and lactic acid fermentation on their presence in milk products]. Dissertation thesis, Veterinary Faculty of University of Ljubljana, Ljubljana
- Cerkvenik V, Doganoc DZ, Skubic V, Beek WMJ & Keukens HJ 2001 Thermal and long-term freezing stability of ivermectin residues in sheep milk. *European Food Research and Technology* **213**(1) 72–76
- Cerkvenik V, Grabnar I, Skubic V, Doganoc DZ, Beek WMJ, Keukens HJ, Drobnič Košorok M & Pogačnik M 2002 Ivermectin pharmacokinetics in lactating sheep. *Veterinary Parasitology* **104** 175–185
- Cinquina AL, Lanzi S, Giannetti L, Fiorucci N, Fagiolo A & Cozzani R 1999 Distribution of ampicillin in goat milk and milk products after a long-acting administration. In *Proceedings of the Euro Food Chem X*, p. 795. Budapest: Federation of European Chemical Societies, Food Chemistry Division
- Courtney CH & Roberson EL 1995 Antinematodal drugs, chemotherapy of parasitic diseases. In *Veterinary Pharmacology and Therapeutics*, 6th edn, pp. 916–932 (Ed. HR Adams). Ames: Iowa State University Press
- Davis M 1991 Ivermectin in an Arkansas dairy herd. *Dairy, Food and Environmental Sanitation* **11**(6) 308
- De Montigny P, Shim JSK & Pivnichny JV 1990 Liquid chromatographic determination of ivermectin in animal plasma with trifluoroacetic anhydride and *N*-methylimidazole as the derivatization reagent. *Journal of Pharmaceutical & Biomedical Analysis* **8**(6) 507–511
- Downing GW 1989 Confirmatory assay for assaying ivermectin residues in liver. In *Ivermectin and Abamectin*, pp. 337–343 (Ed. WC Campbell). New York, USA: Springer-Verlag
- EEC 1990 Council Regulation (EEC) No 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Official Journal of European Community* **L 224** 1–8
- EEC 1993 Council Regulation (EEC) No 2901/93 amending Annexes I, II, III and IV to Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Official Journal of European Community* **L 264** 1–4
- Espósito M, Anastasio A, Imparato E, Fenizia D, Sarnacchiaro T & Serpe L 2001 Parametri farmacocinetici dell'ivermectina nel latte di bufala e valutazione del grado di accumulo nella Mozzarella. *Scienza e tecnica lattiero-casearia* **52**(5) 351–357
- FEDESA 1997 Facts and figures about the European animal health industry. Dossier 14: Animal Health, 16 p. Brussels: FEDESA
- Fletouris DJ, Botsoglou NA, Psomas IE & Mantis AI 1998 Albendazole-related drug residues in milk and their fate during cheese-making, ripening and storage. In *Abstract Book of the 3rd International Symposium on Hormone and Veterinary Drug Residue Analysis*, p. 43. Bruges: University of Ghent, Faculty of Pharmaceutical Sciences
- FSIS 1991 Ivermectin. In *Analytical Chemistry Laboratory Guidebook: Residue Chemistry*, pp. IVR 1–38. Washington, DC: United States Department of Agriculture, Food Safety and Inspection Service (FSIS)
- Lo PKA, Fink DW, Williams JB & Blodinger J 1985 Pharmacokinetic studies of ivermectin: effects of formulation. *Veterinary Research Communications* **9** 251–268
- Longo F, Cinquina AL, Pierdominici E, Raponi A, Fagiolo A, Brambilla G & Cozzani R 1996 Fate of albendazole sulfone and sulfoxide in goat and sheep milk products and by-products. In *Proceedings of the EuroResidue 3. Conference on Residues of Veterinary Drugs in Food*, pp. 654–658. Veldhoven: Federation of European Chemical Societies, Working Party on Food Chemistry
- Miller DJS 1993 Present state and trends in the use of veterinary drugs. In *Proceedings of the EuroResidue 2. Conference on Residues of Veterinary Drugs in Food*, pp. 65–74. Veldhoven: Federation of European Chemical Societies, Working Party on Food Chemistry
- Nordlander I & Johnsson H 1990 Determination of ivermectin residues in swine tissues – An improved clean-up procedure using solid-phase extraction. *Food Additives and Contaminants* **7**(1) 79–82
- Republic of Slovenia 2000 [Regulation of changes and completion of Regulation of veterinary-sanitary control of food plants, veterinary-sanitary examination and of conditions of health suitability of foodstuffs and raw materials of animal origin]. *Uradni list Republike Slovenije [Official Journal of the Republic of Slovenia]* **10**(38) 4534–4535
- Roeder G 1954 [Basis of milking economy and dairying], pp. 689–698. Hamburg and Berlin: Paul Parey
- The Merck Index of chemicals, drugs and biologicals 1989 11th edn, pp. 825–826. Rahway: Merck
- World Health Organisation 1993 Toxicological evaluation of certain veterinary drug residues in food (WHO Food Additives Series 31), pp. 23–35. Geneva: WHO